Failure of Indicator Bacteria to Reflect the Occurrence of Enteroviruses in Marine Waters

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Abstract: The results of several studies conducted along the upper Texas Gulf coast, where a substantial amount of quantitative virological data were collected, are compared to bacteriological indicators and other environmental factors on a statistical basis. Variables common to all these studies were analyzed by multivariate regression. Although multivariate analysis indicated that the number of viruses detected in water was related to rainfall, salinity, and total coliforms in the water, the amount of variation in the number of viruses accounted for by these factors was not large enough to make them good predictors. Enteroviruses were detected 43 per cent of the time in recreational waters considered acceptable as judged by coliform standards, and 44 per cent of the time when judged by fecal coliform standards. Enteroviruses were detected 35 per cent of the time in waters which met acceptable standards for shellfish-harvesting. Our failure to correlate the occurrence of enteroviruses in marine waters with indicator bacteria, and the frequent occurrence of enteroviruses in water which met current bacteriological standards, indicates that these standards do not reflect the occurrence of enteroviruses, and perhaps other human pathogenic viruses, in marine waters. (*Am J Public Health* 69:1116–1119, 1979.)

Introduction

The coastal areas of any nation are a vital recreational and food resource. This valuable resource is in danger of being lost, however, when man overloads the natural self-purification system with his wastes. Human pathogenic enteric viruses (i.e., poliovirus, echovirus, coxsackievirus, infectious hepatitis, etc.) usually occur in domestic sewage and survive in significant numbers even after conventional secondary treatment, including chlorination.[1] They can also survive in seawater and marine sediments for a few days to several weeks,[1] and have been detected in coastal bathing waters.[2] By virtue of their ability to concentrate viruses and bacteria, shellfish such as oysters, mussels and clams may become actively contaminated even at considerable distances from the point of sewage discharge.[3] Shellfish are often eaten raw and have been demonstrated to act as passive carriers of enteric viral disease.[3]

Effective control of enteric bacterial disease spread by recreational waters and shellfish has resulted from the establishment of bacteriological standards using total coliform indices as the basis for limiting recreational use and shellfish harvesting. Much controversy has centered around the adequacy of these standards to reflect a viral disease hazard.[4] This has resulted from the longer survival time of enteric viruses and their resistance to disinfectants (when compared to bacteria) as well as from the relatively low number of viruses necessary to cause an infection.

Enteric viruses have previously been reported in drinking water, marine water, and shellfish which met acceptable bacteriological standards.[1, 3–7] Unfortunately, previous data on the occurrence of enteric viruses in marine waters and shellfish are limited and usually not quantitative. In this report, the results of several studies we conducted along the upper Texas Gulf coast, where a substantial amount of quantitative virological data was collected, are compared to bacteriological indicators and other environmental factors on a statistical basis. It was hoped that such analysis would provide information on the relative importance of environmental factors influencing the presence of enteric viruses in marine waters and help establish a quantitative basis for the relationship between indicator bacteria and the presence of enteric viruses in marine waters.

Materials and Methods

Over a period of three years, 150 water samples were obtained from 26 sites along the Galveston Bay area, located near Houston, Texas, on the upper Texas Gulf coast. Samples were collected from coastal canal communities and undeveloped shore sites bordering the bay as well as from open bay water several miles from shore. A detailed description of the sites can be found in previous studies reported by this

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laboratory.[3, 5, 6] For the virus studies, 400-liter samples were processed.

The isolation and enumeration of coliform, fecal coliform, and enteroviruses from water and sediment were carried out using previously described methodology.[3, 5, 6] Salinity, turbidity, pH, temperature, and the suspended solids determinations have also been described in detail.[3, 5, 6]

Results

The statistical evaluation of the relationship between the number of viruses and the environmental variables was performed through multivariate regression analysis.[8] Prior to this analysis, scattergrams of the viral counts with each independent variable were made and examined. This step proved to be very helpful in the search for the best mathematical approximation of the complex process studied. For all the variables (except for rain index, which was a discrete variable), the bivariate relationships with the viral count as an independent variable were approximated by a least squares fit of a linear model. The possibility that an exponential curve may provide better approximation of the biological process under study was also considered and thus, the least squares fit of the log-transformed data was examined.

The number of viruses in the water, denoted by y, can be regarded as a function of various characteristics of water quality and sediments, denoted by $x_1, x_2 \ldots x_8$. The hypothesized functional dependence

$$y = f(x_1; x_2; x_3; \ldots x_8) \qquad (1)$$

was studied by stepwise multiple regression (SPSS computer package, version 7).[9] The results of this analysis are shown in Table 1.

The variables for which regression coefficients were found to be statistically significant ($0.05 < P \leq 0.01$) were: 1) rainfall, 2) salinity, and 3) the number of total coliforms in water. First factor, the presence or absence of rain, accounted for approximately 9 per cent of variance in the number of viruses detected in water. The addition of two other variables—salinity and the number of total coliforms in water—added 3 and 4 per cent, respectively, to R^2 value and increased the amount of explained variance to about 16%. Thus, an association between viruses and some environmental factors exists, however small, and there is less than a 5 per cent chance that it was found by accident.

The addition of the five remaining variables yielded an increase in the R^2 of the 8 variable model by only 1 per cent. The product-moment correlation matrix (Table 2) shows that there is some interdependence between variables, i.e., the numbers of total and fecal coliforms in water and sediment correlate significantly with water salinity and to some extent with the presence or absence of rain and with pH.

Discussion

In this report, the combined results of several studies on the occurrence of enteroviruses in marine waters have been analyzed. Only those variables common to all of the previous studies have been considered. Each of the studies was limited in scope and was conducted in a limited geographical area. Thus, the data analyzed here represent a wide range of marine environments (i.e., shallow waterways, open bay waters, etc.), and include areas both heavily polluted and nonpolluted as judged by bacteriological indicators. Study sites included both recreational areas and areas open to shellfish harvesting. The analyses were performed on the largest amount of quantitative virological data that has been reported for marine waters.

Although the multivariate regression analysis showed that the number of viruses detected in water correlated significantly with the number of total coliforms in water, the amount of variation in the number of viruses accounted for by this factor (4 per cent) was not large enough to make it a good predictor. Other bacterial indicators were not found to be statistically significant.

Without epidemiological data, it is difficult to assess the meaning and importance of the possible failure of indicator bacteria to reflect a viral disease hazard. Epidemiological studies to establish a relationship between viral disease and the presence of viruses in water would be a formidable task, and might not yield meaningful results, because clinically observable illness occurs only in a small number of people who become infected and because of the widely varying incubation periods.[10] This consideration, as well as the low

TABLE 1—Multiple Regression of Viruses in Water and Other Water Quality Indicators

Variable	Significance of Each Variable	Multiple R	R-Square	R-Square Change	Simple R	Overall F	Overall Significance of the Model
Rainfall	.001	.30240	.09145	.09145	.30240	10.76953	.001
Salinity	.046	.35365	.12507	.03363	.09340	7.57634	.001
TCW*	.029	.40492	.16396	.03889	.26789	6.86385	.000
FCW*	.428	.41113	.16903	.00507	.16373	5.28860	.001
pH	.623	.41350	.17098	.00196	−.19038	4.24872	.001
Turbidity	.601	.41619	.17321	.00223	−.02830	3.56145	.003
FCS*	.645	.41828	.17496	.00175	.01412	3.05971	.006
TCS*	.887	.41848	.17512	.00017	.08343	2.65379	.011

*TCW, FCW, FCS, TCS = number/100 ml of total coliforms in water, fecal coliforms in water, fecal coliforms in sediment, and total coliforms in sediment, respectively.

TABLE 2—Product-Moment Correlation Matrix of All Measured Variables

Variable	pH	Salinity	Turbidity	Rain	TCW	TCS	FCW	FCS	Virus
pH	1.0000								
Salinity	0.1149	1.0000							
Turbidity	−0.0173	−0.1368	1.0000						
Rain	−0.2400	−0.1399	−0.0835	1.0000					
TCW*	−0.5461†	−0.4902†	−0.0064	0.5066†	1.0000				
TCS*	−0.2367	−0.3532†	−0.1165	0.1793	0.4112†	1.0000			
FCW*	−0.3476	−0.2584†	−0.0572	0.41442†	0.6822†	−0.0152	1.0000		
FCS*	−0.0974	−0.2194‡	−0.0947	0.0831	0.1441	0.1627	0.1460	1.0000	
Virus	−0.1159	0.0992	−0.0176	0.2830†	0.2476§	0.0834	0.1508	0.0155	1.0000

*TCW, TCS, FCW, FCS = no./100 ml of total coliforms in water, total coliforms in sediment, fecal coliforms in water, and fecal coliforms in sediment, respectively.
†Significant ($P < 0.01$).
‡Significant ($P < 0.02$).
§Significant ($P < 0.03$).

infective dose of viruses,[11] has led some to suggest that the presence of enteric viruses in any water is indicative of a potential viral disease hazard.[12]

The need for standards governing the sanitary quality of marine waters used for recreation has been recognized by public health officials for many years. In response to this need, most states have adopted standards based on federal recommendations for the sanitary quality of waters used for recreational bathing and shellfish harvesting. Total and fecal coliform bacteria are in general use in the United States for judging the acceptability of contact recreational waters and shellfish-harvesting waters. Water is generally not considered acceptable for contact recreation when the total and fecal coliform densities of 1000 and 200 per 100 ml, respectively, are exceeded.

These standards were based on epidemiologic studies using Lake Michigan and the Ohio River, where detectable health effects were associated with a total coliform density of 2000/100 ml. This was extrapolated to a fecal coliform density of 400/100 ml. This value was reduced to 200/100 ml on the assumption that the quality of direct contact recreational waters should be better than that which produced a demonstrable health effect.[13]

Such standards have been applied universally to both marine and fresh water bathing areas. More recent epidemiological studies among swimmers at marine bathing beaches have also indicated a relationship between indicator bacteria and gastrointestinal illness.[14] The Environmental Protection Agency is currently conducting a program to develop such water quality criteria for recreational waters.[15] The result of these findings thus far is that a swimming-associated gastroenteritis, primarily in children, can be quantitatively associated with the quality of the bathing water as measured by *Escherichia coli* or enterococcus densities. The gastroenteritis typically has a short incubation period, an acute onset, a short period of relatively benign symptoms, and no sequelae, although in some individuals the symptoms were disabling enough for them to remain home, remain in bed, or seek medical advice. The association between illness and the presence of as few as 10 *E. coli* per 100 ml suggests that the agent(s) responsible for the observed illness is (are) highly infectious, is (are) present in sewage in large numbers and/or survives much longer than *E. coli* in the marine environment. These characteristics, along with the nature of the illness, suggest a viral etiology, probably related to rotavirus or parvo-like viruses.

Because of their ability to concentrate bacteria and viruses from water during feeding, there is a greater potential risk associated with shellfish consumption than with recreational use of the same waters. The present microbiological standard in the United States for shellfish-harvesting waters requires a median of 70 coliforms per 100 ml, with no more than 10 per cent of the samples exceeding a value of 230.[16] Enforcement of this standard has resulted in the absence of shellfish-associated typhoid in this country since 1959.[13] However, outbreaks of shellfish-associated infectious hepatitis and nonspecific gastroenteritis continue to occur.[3,16] The most noted recent outbreak was of infectious hepatitis associated with the consumption of raw oysters in Texas, Louisiana, and Georgia, in which the oysters were harvested from waters meeting national sanitation standards and which were certified for oyster harvesting.[16]

In the combined studies considered in this report, viruses were detected 43 per cent of the time in recreational waters considered acceptable as judged by coliform standards, and 44 per cent of the time when judged by fecal coliform standards. Viruses were detected in waters which met acceptable standards for shellfish harvesting 35 per cent of the time. Our failure to correlate the occurrence of viruses in marine waters with indicator bacteria, and their occurrence with high frequency in waters which met current bacteriological standards, indicates that these standards do not reflect the occurrence of enteroviruses in marine waters.

Recently, standards have been proposed for viral quality of recreational water. Melnick[17] recommended consideration of a limit of one infectious unit of virus per 10 gallons of recreational water. This standard was exceeded in about one-third of the samples referred to in the present study. Because of the lack of epidemiologic data, such a standard is arbitrary and reflects limitations of current detection methodology for enteric viruses in water rather than disease risk. They may be conservative since the efficiency of concentration methodology of enteroviruses averages 50 per cent, and current concentration and detection methods are optimized for enteroviruses and not capable of recovering rotaviruses, infectious hepatitis virus, adenoviruses, or the Norwalk agent (which may also be present in wastewater discharges[18]); in fact, current methodology is only optimized for detection of less than 40 per cent of the enteric viruses which could be present in sewage-contaminated waters.

Of the environmental factors examined, the only ones for which a clear relationship with virus isolation could be demonstrated were the occurrence of rainfall within 24 hours of sampling and salinity of water. There was also some indication that a logarithmic correlation existed between the presence of virus and turbidity, which was probably influenced by the rainfall. The increase of virus in water after periods of heavy rainfall could result from disturbance of sediments containing viruses,* runoff, or flushing of sewage-laden waterways which empty into the bay. Although the statistical model, which estimates the number of viruses detected in water based on three factors—presence or absence of rain, water salinity, and number of total coliforms in water—fits well to the observed data, the amount of variance described by this model is only about 16 per cent. Thus a large amount of variance remains unexplained and is likely to be due to other factors not considered here. Such variance could be related in part to errors encountered in the measurement of the parameters studied, i.e., sampling variations due to differences in the efficiency of virus detection from one sample to another.

The effect of environmental factors controlling enteric viruses in marine water may be greatly influenced by geophysical parameters (i.e., bottom topography, shoreline contours, water depth, inflow changes, etc.), in that it may be difficult to apply findings of this study to other coastal areas. Clearly, more work is needed on factors controlling the occurrence of viruses in marine waters for the effective management of marine water quality.

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